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A 3-SNP gene risk score and a metabolic risk score both predict hypertriglyceridemia and cardiovascular disease risk

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BACKGROUND: Evidence on the causal link between plasma triglyceride (TG) levels and risk for cardiovascular disease (CVD) has recently emerged. Individuals with the metabolic syndrome have an increased risk for acquiring elevated TG levels later in life. Moreover, common DNA sequence variations in genes affecting TG levels identify individuals at risk for elevated plasma TG levels.

OBJECTIVE: We evaluated whether a 3-single nucleotide polymorphism (SNP) TG gene risk score (GRS) and a metabolic risk score (MetRS) both improved CVD risk prediction.

METHODS: A 3-SNP GRS and MetRS were generated in the EPIC-Norfolk cohort (n = 20,074) based on 3 SNPs in *LPL* and *APOA5* or the number of Metabolic Syndrome criteria present (maximum 5), respectively. The associations between the 3-SNP GRS, MetRS, TG levels, and CVD risk were evaluated.

RESULTS: The 3-SNP GRS and MetRS were both linearly associated with plasma TG levels, that is, +0.25 mmol/L [95% CI 0.22–0.27] per allele change ($P < .001$) and +0.72 mmol/L [95% CI 0.70–0.73] per increase of number of metabolic syndrome risk score points ($P < .001$), respectively. We observed a positive association between the 3-SNP GRS and the risk of CVD with an adjusted hazard ratio (HR) of 1.35 [95% CI 1.04–1.74] for the highest versus the lowest GRS, which was independent of the MetRS. For the MetRS, the adjusted HR was 2.03 [95% CI 1.73–2.40] for the highest versus the lowest MetRS.

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CONCLUSION: Both the 3-SNP GRS and the MetRS are associated with increased plasma TG levels and increased risk for CVD.

Introduction

Hypertriglyceridemia, that is, an elevated plasma level of triglycerides (TG), is a trait that is influenced by numerous factors like lifestyle, environmental factors, and genetic predisposition. The importance of TG for cardiovascular disease (CVD) risk estimation has emerged since 2007 from large epidemiological studies, genetic studies, and clinical trials, showing a consistent and robust association.[1–10] TGs are derived from dietary sources or from direct hepatic de novo lipogenesis. Exogenous TG is transported in apo-B48–containing chylomicron particles that undergo a rapid hepatic catabolism. Apo B-100–containing VLDL particles of hepatic origin are the major circulating TG-carrying lipoproteins that on entering the circulation undergo rapid lipoprotein lipase (LPL)-mediated lipolysis allowing free fatty acid uptake in tissues for energy use or storage, which eventually results in the generation of cholesterol-rich LDL particles. In the presence of reduced LPL-mediated lipolysis, elevated fasting plasma TG levels are a marker for the presence of increased circulating cholesterol- and TG-enriched apo-B100–containing remnant particles, that is, very low density and intermediate density lipoproteins that are associated with increased risk for CVD.[11–15]

Over the years, several common single nucleotide polymorphisms (SNPs) with significant effects on plasma TG levels have been identified, including genetic variants in lipoprotein lipase (*LPL*) and apolipoprotein A-V (*APOA5*).[16–18] Subsequently, genome-wide association studies (GWAS) were able to replicate these findings and identify additional loci, such as those encoding for tribbles pseudokinase 1(*TRIB1*), glucokinase regulator (*GCKR*), and angiopoietin-like protein 4 (*ANGPTL4*), thereby stressing the polygenic nature of plasma TG levels.[4,19–28] It has been shown that loss of function variants in *APOC3* results in decreased risk for CHD as shown in 2 large population cohorts.[29–32] Thus, Mendelian randomization studies support the concept that the association between TG levels and CVD risk exists, however, causality remains to be assessed.[33–36]

Elevated plasma TG levels are frequently observed in metabolic syndrome patients,[37] besides other metabolic abnormalities such as low high-density lipoprotein cholesterol (HDL-C), hypertension, obesity, and type 2 diabetes mellitus. However, hypertriglyceridemia may additionally occur because of the presence of loss of function variations in genes that play an essential role in TG metabolism.[36] A similar observation has been met with regard to the presence of elevated plasma low-density lipoprotein cholesterol (LDL-C) levels. Genetically determined low levels of LDL-C were shown to be more strongly associated with low cardiovascular disease (CVD) risk than short-term statin-mediated lowering of plasma LDL-C levels.[38]

We hypothesize that a 3-SNP gene risk score (GRS) based on genetic variants influencing plasma TG will be associated with increased risk for CHD. In the present study, we set out to test which variants should be used in a 3-SNP GRS. Next, we investigated the relationship of the 3-SNP GRS and a clinically derived metabolic risk score (MetRS) as risk factors for CVD in the European Prospective Investigation of Cancer (EPIC)-Norfolk prospective population study.

Methods

Study design

The EPIC-Norfolk prospective population study consists of 25,639 individuals recruited from general practices in the Norfolk area, United Kingdom.[39] Study participants aged between 39 and 79 years were enrolled between 1993 and 1997. At baseline, patients completed general health and lifestyle questionnaires. During follow-up, all participants were flagged for mortality at the UK Office of National Statistics, and vital status was ascertained for the entire cohort. Data on all hospital contacts throughout England and Wales were obtained using National Health Service numbers through linkage with the East Norfolk Health Authority (ENCORE) database. Hospital records and death certificates were coded by trained nosologists and categorized according to the International Classification of Disease 10th (ICD-10) revision. Death or hospitalizations were attributed to an outcome if the corresponding ICD-10 code was recorded as the underlying cause. The study protocol was approved by the Norwich District Health Authority Ethics Committee. All participants gave written informed consent. In the complete EPIC-Norfolk cohort, DNA-SNP data were available for n = 20,074 individuals.

In 2004, a prospective case-control substudy was designed including participants of the EPIC-Norfolk study. Cases were people who developed CAD during follow-up until 2003. Control participants remained free of any CVD during an average of 7.4 years of follow-up. The controls were matched to each case by sex and age.[40] In this substudy, DNA from n = 2649 participants (2132 controls and n = 2105 cases) was available for further analyses as well as plasma samples for in depth phenotyping.

Biochemical analysis

Nonfasting blood samples were taken by venipuncture in EDTA-containing vacutainers at the baseline clinic visit and processed at the Department of Clinical Biochemistry, University of Cambridge (UK), where all lipids assays were performed.[41] Plasma was isolated by centrifugation for 15 minutes, 3000 rpm, 4°C, and stored at −80°C for future analysis. Circulating levels of total cholesterol, HDL-C, and TG were measured on fresh samples with the RA 1000 (Bayer Diagnostics, Basingstoke, United Kingdom), and LDL-C levels were calculated with the Friedewald formula.[42] Non-HDL cholesterol was calculated by subtracting HDL-C from total cholesterol. Apo B was measured by using rate immunonephelometry (Behring Nephelometer BNII, Marburg, Germany).[43] HbA1c was measured using Diamat ion exchange HPLC (Bio-Rad Laboratories, Hemel Hempstead, United Kingdom).[39]

In the case-control subset, additional parameters were tested. Lipoprotein particle size and particle number was analyzed with an automated nuclear magnetic resonance (NMR) spectroscopy by Liposcience Co as described.[44] Plasma LPL mass and Apo A-V were measured as described.[16] Samples were analyzed in random order to avoid systemic bias. Researchers and laboratory personnel had no access to identifiable information and could identify samples by number only.

SNP selection and genotyping on the case/control cohort

We selected SNPs based on their association with elevated plasma TG levels. A number of different sources were used: (1) leading SNPs significantly associated with plasma TG levels identified in a large GWAS study (*LPL and APOA5*),[28] (2) common SNPs, which were originally published in association studies (*LPL, APOA5, APOA4, ANGPTL4, GALNT2, APOC3, GCKR, TRIB1*)[20,28,45–48], and (3) common variants at the *APOC2, LMF1,* and *GPIHBP1* loci that repeatedly show no significant association with plasma TG in large GWAS.[20,45–48] Patients with complete loss of function mutations in these 3 genes suffer from a monogenic form of severe hypertriglyceridemia, resembling complete or partial LPL deficiency. No data are available to date that show the influence of common genetic variants in these genes and the effect on plasma TG levels in the general population.[48] Tagging SNPs were selected as described previously using the public available Hapman program.[40] Genotyping was performed using allelic discrimination with VIC- and FAM-labeled probes designed by Applied Biosystems (Thermo Fisher Scientific Inc, Waltham, MA). PCR conditions were denaturation for 10 min at 95°C, followed by 40 cycles (30 sec 92°C, 45 sec 60°C) and run on a CFX PCR system (BioRad Laboratories Inc, CA). Taqman PCR assay mix was obtained from Applied Biosystems (Thermo Fisher Scientific Inc, Waltham, MA). All SNPs were initially determined in the random case-control subset of the cohort (n = 2649). SNPs that were significantly associated with plasma TG levels ($P < .05$) were selected and tested in a backward elimination model to explore which SNPs appeared to drive the association with plasma TG levels. The SNPs, which remain associated with plasma TG levels, were used to create the 3-SNP GRS. For each TG-raising allele 1 point was given. Genotyping in the complete cohort was performed using the GeneChip Human Mapping 500K Array Set from Affymetrix (Santa Clara, CA).

Metabolic risk score (MetRS)

The presence and severity of the metabolic syndrome was determined according to the harmonized criteria for the metabolic syndrome.[49] To meet the threshold for metabolic syndrome, participants had to present with 3 or more of the following conditions: (1) waist circumference ≥94 cm for men or ≥80 cm for women, (2) TG ≥ 150 mg/dL (1.7 mmol/L), (3) HDL-C <40 mg/dL (1.03 mmol/L) in men or <50 mg/dL (1.29 mmol/L) in women, (4) systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥85, or treatment for hypertension, and/or (5) fasting plasma glucose ≥100 mg/dL (5.6 mmol/L) or previously diagnosed type 2 diabetes. Because fasting plasma glucose levels were not available in the cohort, HbA1c was chosen as a surrogate for fasting plasma glucose, with a cutoff of ≥5.6%.[50] A composite MetRS was generated based on the presence of the number of metabolic syndrome criteria.

Cardiovascular outcomes definition

For cardiovascular outcomes, the definition of the ACC/AHA was used, which included fatal or nonfatal myocardial infarction or coronary heart disease (CHD) or fatal or nonfatal stroke.[51] A composite of the following ICD-10 codes: I20-25 and I60-69 was assessed. As no ICD-10 definition of stroke was reported in the guideline, the definition previously used by the AHA (comprising ICD-10 codes I60-69) was used.[52]

Statistical analysis

The MetRS was calculated for all people in the complete cohort. The relationship between single SNPs and plasma TG levels was assessed by a linear regression model. Variables with skewed distribution were log-transformed before being used as continuous variables in statistical analyses. Associations between GRS and plasma lipid parameters were calculated using linear regression for continuous variables and the chi-square test for trend for categorical variables. To estimate the relative risk of CVD, a Cox proportional hazards model was used to calculate hazard ratios (HRs) and 95% confidence intervals for the GRS, using the lowest TG GRS as the reference category. For the GRS, multivariable adjustment was used, correcting for age, sex, BMI, diabetes, smoking status, and

Table 1 Plasma triglyceride concentration according to triglyceride-related SNPs

Gene	SNP	N	GT	TG (mmol/L)	GRS points per SNP	mmol/L TG increase per allel	P^*	$P^\dagger$
APOA5	rs3135506	17,555	CC	1.50 (1.10–2.20)	0	0.28	<.001	<.001
		2432	CG	1.70 (1.20–2.50)	1			
		87	GG	1.90 (1.40–3.30)	2			
APOA5	rs662799	17,918	AA	1.50 (1.10–2.20)	0	0.29	<.001	<.001
		2077	AG	1.70 (1.20–2.50)	1			
		79	GG	1.80 (1.20–3.10)	2			
LPL	rs328	16,095	CC	1.60 (1.10–2.30)	2	0.19	<.001	<.001
		3762	CG	1.40 (1.00–2.00)	1			
		217	GG	1.30 (0.90–1.90)	0			

Number of individuals (N) and triglyceride serum concentrations according to polymorphism are presented as median and 25th and 75th percentile. GRS point were used for the calculation of the total 3-SNP GRS.

GRS = gene risk score; GT = genotype; P^* = unadjusted *P*-value, $P^\dagger$ = *P*-value adjusted for age, sex, waist, body mass index, and diabetes. Published frequency of the SNPs: rs3135506: 0.067; rs662799: 0.8997; rs328: 0.094.

LDL-cholesterol. For the MetRS, HRs were calculated using the group with without the metabolic syndrome as the reference category. Multivariable adjustment was used, correcting for age, sex, and smoking.

HRs for CVD were calculated for participants with respect to their 3-SNP GRS, stratified for the presence or absence of the metabolic syndrome (MetRS ≥3/5, MetRS ≤2/5, respectively). The category with a 3-SNP GRS of 0/6 and a MetRS of ≤2/5 was used as the reference category. A probability value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS (Version 21.0, IBM Corporation, Armonk, NY).

Results

Selection of SNPs for GRS

We determined leading GWAS SNPs in *TRIB1, GCKR, APOA5, LPL, APOC2, APOC3*, known missense variants in *ANGPTL4* and *APOA4* and tagSNPs in *LMF1* and *GPIHBP1* in a random subset of the EPIC-Norfolk cohort (n = 2649). The biochemical characteristics of the subset case-control cohort are presented in Table S2. A higher BMI and a worsened lipid profile were present in the cases as compared to the age-matched controls. Details of all SNPs are documented in Table S1. We did not find any associations between genetic variations in *LMF1, APOC2, APOA4, GCKR, APOC3, GALNT2,* or *GPIHBP1* and plasma TG levels. The SNPs in *ANGPTL4* (rs1044250, rs116843064), *TRIB1* (rs2954029), *LPL* (rs328, rs12678919), and *APOA5* (rs75423577, rs3135506, rs662799) were all significantly associated with plasma TG levels with *P* values varying from <.05 to <.001 (Table S3). To explore which SNPs appear to drive the association with plasma TG levels, we used backward elimination in a model that included the 8 SNPs that were associated with plasma TG levels. Only 3 SNPs remained significantly associated with plasma TG levels (rs3135506 and rs662799 in *APOA5* and rs328 in *LPL*) and were therefore selected to generate the 3-SNP GRS (Table S3). The

Table 2 Demographic and lifestyle parameters of individuals according to the 3-SNP GRS

3-SNP GRS	0/6	1/6	2/6	3/6	≥4/6	*P*
N	157	2971	13,284	3394	268	
Men, n (%)	78 (50%)	1381 (46%)	6203 (47%)	1584 (47%)	124 (46%)	.96
Age, y	59.5 ± 9.3	59.0 ± 9.3	59.2 ± 9.3	59.0 ± 9.3	58.7 ± 9.2	.41
Body mass index, kg/m^2	26.4 ± 3.7	26.3 ± 3.8	26.3 ± 3.8	26.3 ± 3.8	26.3 ± 3.9	.98
Waist circumference, cm	88.5 ± 11.8	88.4 ± 12.5	88.3 ± 12.3	88.4 ± 12.2	87.3 ± 12.1	.75
Current smoker, n (%)	16 (10.3)	325 (11.1)	1534 (11.6)	374 (11.1)	33 (12.4)	.86
Diabetes mellitus, n (%)	4 (2.6)	59 (2.0)	279 (2.1)	88 (2.6)	8 (3.0)	.34
Systolic blood pressure, mmHg	136 ± 18	135 ± 18	135 ± 18	135 ± 18	138 ± 19	.15
Diastolic blood pressure, mmHg	82 ± 11	82 ± 11	82 ± 11	83 ± 11	84 ± 11	.10
Mean MetRS	0.32	0.43	0.49	0.57	0.68	

Data are presented as number (percentage), mean ± SD.

MetRS = metabolic risk score, 3-SNP GRS = 3-single nucleotide polymorphism gene risk score.

Table 3 Distribution of plasma lipids according to the 3-SNP GRS

3-SNP GRS	0/6	1/6	2/6	3/6	≥4/6	R	P^*	$P^\dagger$
Participants, n (%)	157 (0.8)	2971 (14.8)	13,284 (66.2)	3394 (16.9)	268 (1.3)			
Triglyceride, mmol/l	1.30 (0.90–1.90)	1.40 (1.00–2.00)	1.50 (1.10–2.20)	1.70 (1.20–2.50)	2.10 (1.30–3.20)	0.143	<.001	<.001
Total cholesterol, mmol/L	6.12 ± 1.02	6.12 ± 1.13	6.16 ± 1.14	6.27 ± 1.24	6.46 ± 1.37	0.046	<.001	<.001
HDL-cholesterol, mmol/L	1.58 ± 0.39	1.48 ± 0.42	1.41 ± 0.42	1.38 ± 0.47	1.27 ± 0.36	−0.080	<.001	<.001
LDL-cholesterol, mmol/L	3.87 ± 0.92	3.92 ± 1.01	3.95 ± 1.02	4.01 ± 1.08	4.11 ± 1.14	0.028	<.001	<.001
Non-HDL-cholesterol, mmol/L	4.50 ± 1.08	4.62 ± 1.14	4.71 ± 1.15	4.83 ± 1.24	5.05 ± 1.27	0.062	<.001	<.001
Lipoprotein(a), mg/dl	12.80 (5.16–25.15)	12.02 (6.33–27.15)	11.48 (6.16–27.30)	11.171 (5.87–26.64)	10.96 (5.60–25.47)	−0.002	.82	<.001
ApoB, g/L	0.91 ± 0.23	0.96 ± 0.24	0.97 ± 0.24	0.98 ± 0.25	1.00 ± 0.27	0.029	<.001	<.001

Data are presented as number (percentage), median and 25th and 75th percentile or mean ± SD, 3-SNP GRS = 3-single nucleotide polymorphism. R = Pearson or Spearman correlation, P^* = unadjusted *P*-value, $P^\dagger$ = *P*-value adjusted for age, sex, diabetes, waist, and body-mass index.

SNPs in *APOA5* were in linkage disequilibrium, but to better discriminate the different groups both SNPs were used. The 3-SNP GRS included the number of TG-raising alleles for each of the selected SNPs carried by each participant. Scores were given for each TG-raising allele (Table 1). In GRS 0/6 are those individuals that do not have any of the variants; in GRS-1/6 are the carriers heterozygous for the *LPL* variant; in GRS 2/6 the carriers who are heterozygous for the *APOA5* variants and carriers homozygous for the *LPL* variant; in GRS 3/6 are carriers heterozygous for the *LPL* as well as the *APOA5* variants, whereas GRS ≥4/6 harbors the rest of the individuals.

Associations between the 3-SNP GRS and lipid parameters are displayed in Table S4. Across the GRS categories, we observed a GRS-dependent increase toward an atherogenic lipoprotein profile. Plasma apoA-V and apo B levels were significantly increased, whereas plasma LPL mass was significantly reduced. Plasma levels of total cholesterol and LDL-C were significantly increased in the higher GRS categories, whereas HDL-C levels were significantly decreased (all $P < .001$). Interestingly, a higher 3-SNP GRS coincided with the presence of smaller LDL particle size and greater LDL particle number ($P < .001$). Inline, VLDL particle number was significantly increased ($P < .001$). Concomitantly HDL particles size was smaller but no difference in HDL particle number was observed.

Association between the 3-SNP GRS and plasma TG levels in the complete EPIC cohort

The 3-SNP GRS was evaluated in n = 20,074 participants from the EPIC-Norfolk cohort with complete SNP data available. All 3 SNPs were in Hardy–Weinberg equilibrium with a probability value of >0.05. Carriership of the rs3135506 G allele, rs662799 G allele (both in *APOA5*), and rs328 C allele (in *LPL*) was associated with

Table 4 Demographic and lifestyle parameters according to the MetRS

Metabolic risk score	≤2/5	3/5	4/5	5/5	*P*
N	13,113	4283	2316	362	
Men, n (%)	5529 (42)	2337 (55)	1304 (56)	200 (55)	<.001
Age, y	57.7 ± 9.2	61.4 ± 8.9	62.3 ± 8.6	63.9 ± 8.3	<.001
Body mass index, kg/m^2	25.1 ± 3.3	28.1 ± 3.6	29.2 ± 3.7	30.1 ± 4.2	<.001
Waist circumference, cm	83.9 ± 10.9	94.9 ± 10.4	98.7 ± 10.1	101.5 ± 11.8	<.001
Current smoker, n (%)	1466 (11.3)	486 (11.4)	279 (12.2)	51 (14.1)	<.001
Diabetes mellitus, n (%)	715 (5.8)	754 (21.4)	767 (49.5)	362 (100%)	<.001
Systolic blood pressure, mmHg	131 ± 17	142 ± 17	145 ± 16	146 ± 17	<.001
Diastolic blood pressure, mmHg	80 ± 11	87 ± 11	88 ± 10	88 ± 10	<.001
Mean 3-SNP GRS	2.0	2.1	2.1	2.1	

Data are presented as number (percentage), mean ± SD. 3-SNP GRS = 3-single nucleotide polymorphism gene risk score.

Table 5 Distribution lipoproteins according to the MetRS

Metabolic risk score	≤2/5	3/5	4/5	5/5	R	P^*	$P^\dagger$
Participants, n (%)	13,113 (65.3)	4283 (21.3)	2316 (11.5)	362 (1.8)			
Triglyceride, mmol/l	1.30 (0.90–1.60)	2.10 (1.70–2.90)	2.50 (2.10–3.20)	2.70 (2.20–3.40)	0.500	<.001	<.001
Total cholesterol, mmol/L	6.01 ± 1.10	6.50 ± 1.22	6.48 ± 1.20	6.30 ± 1.19	0.164	<.001	<.001
HDL-cholesterol, mmol/L	1.54 ± 0.40	1.25 ± 0.40	1.05 ± 0.25	0.96 ± 0.15	−0.435	<.001	<.001
LDL-cholesterol, mmol/L	3.85 ± 1.00	4.19 ± 1.04	4.19 ± 1.08	4.00 ± 1.14	0.129	<.001	<.001
Non-HDL-cholesterol, mmol/L	4.45 ± 1.09	5.17 ± 1.13	5.39 ± 1.13	5.33 ± 1.17	0.310	<.001	<.001
Lipoprotein(a), mg/dl	11.6 (6.2–28.3)	11.2 (6.1–25.2)	11.3 (5.7–25.0)	12.2 (5.51–28.22)	−0.022	.006	<.001
Apo B, g/L	0.94 ± 0.23	1.03 ± 0.25	1.02 ± 0.87	1.00 ± 0.26	0.156	<.001	<.001

Data are presented as number (percentage), median and 25th and 75th percentile or mean ± SD.
R = Pearson or Spearman correlation, P^* = unadjusted *P*-value, $P^\dagger$ = *P*-value adjusted for age, sex.

higher plasma TG concentrations (+0.28 mmol/l [95% CI 0.23–0.32] per rs3135506 G allele; +0.29 mmol/l [95% CI 0.24–0.33] per rs662799 G allele and +0.19 mmol/l [95% CI 0.15–0.22] per rs328 C allele) (Table 1). The demographic and lifestyle parameters of individuals according to the 3-SNP GRS, ranging from 0/6 to ≥4/6, are shown in Table 2. In total, 157 participants had a GRS of 0/6, whereas 268 participants had a GRS of ≥4/6. No significant differences between age, sex, body mass index (BMI), waist circumference, smoking status, presence of type 2 diabetes mellitus, or blood pressure were observed between the GRS groups. In Table 3 the distribution of plasma TG, cholesterol, and apo B levels according to the 3-SNP GRS is displayed. The groups with the highest 3-SNP GRS score have a less favorable lipid phenotype. Based on GRS, TG levels ranged from 1.30 (0.90–1.90) to 2.10 (1.30–3.20) for the lowest (0/6) and the highest (≥4/6) 3-SNP GRS, respectively, and were statistically significant different ($P < .001$). Total cholesterol ranged from 6.12 ± 1.02 to 6.46 ± 1.37 mmol/l, HDL-C from 1.58 ± 0.39 to 1.27 ± 0.36 mmol/l, LDL-C from 3.87 ± 0.92 to 4.11 ± 1.14 mmol/L, and Apo B ranged from 0.91 ± 0.23 to 1.00 ± 0.27 g/L.

Association of MetRS with plasma TG levels

Next, participants were classified in 4 different categories according to their MetRS (from ≤2/5 ie, no metabolic syndrome to 5/5 being most severely affected). As expected, significant differences ($P < .001$) were observed between the MetRS groups for sex, age, BMI, waist circumference, smoking status, diabetes mellitus, and blood pressure (Table 4). Plasma TG levels ranged from 1.30 (0.90–1.60) to 2.70 (2.20–3.40) mmol/L in the group with the lowest (≤2/5) and the highest (5/5) MetRS ($P < .001$). Inline, total cholesterol levels ranged from 6.01 ± 1.10 to 6.30 ± 1.19 mmol/L, HDL-C ranged from 1.54 ± 0.40 to 0.96 ± 0.15 mmol/L, LDL-C ranged from 3.85 ± 1.00 to 4.00 ± 1.14 mmol/L in the group with the lowest (≤2/5) and the highest (5/5) group, suggesting the presence of the most unfavorable lipid phenotype in MetRS group with score 5 (Table 5).

In the small case-control subset, MetRS was associated with a similar unfavorable lipid profile as observed for the 3-SNP GRS, including significant differences in plasma apo A-V, apo B, and LPL mass as well as unfavorable changes in VLDL and particle size and number.

Association of the 3-SNP GRS and MetRS with CVD risk

We assessed the hazard ratios associated with future CVD per 3-SNP GRS and MetRS category (Tables 6 and 7, respectively). Mean follow-up was 16 years. With respect to the 3-SNP GRS, the unadjusted hazard ratio (HR) for individuals in the highest category (GRS ≥4/6) was 1.35

Table 6 Risk of future coronary artery disease and stroke per 3-SNP GRS category

3- SNP GRS	≤1/6	2/6	3/6	≥4/6	$P^\dagger$
Cases (%)/controls	669 (21%)/2459	3169 (24%)/10,115	776 (23%)/2618	77 (29%)/191	
HR (95% CI)	1.00	1.14 (1.05–1.23)	1.07 (0.97–1.19)	1.35 (1.07–1.71)	.005
P-value		.003	.178	.012	
HR* (95% CI)	1.00	1.14 (1.05–1.24)	1.07 (0.96–1.19)	1.36 (1.07–1.72)	<.001
P-value*		.002	.207	.011	
HR‡ (95% CI)	1.00	1.12 (1.03–1.22)	1.04 (0.93–1.15)	1.35 (1.04–1.74)	<.001
P-value‡		.010	.495	.024	

Hazard ratio (HR) and 95% CI, relative to TG gene score ≤1, for each 3-SNP GRS = 3-single nucleotide polymorphism gene risk score category.
*HR adjusted for age, sex, body-mass index, diabetes, and smoking with corresponding *P*-value.
†*P* value for linearity.
‡HR adjusted for age, sex, body mass index, diabetes, smoking, and LDL-C with corresponding *P*-value.

Table 7 Risk of future coronary artery disease and stroke per MetRS category

Metabolic risk score	≤2/5	3/5	4/5	5/5	$P^{\dagger}$
Cases (%)/controls	2358 (18%)/10,755	1351 (32%)/4283	828 (36%)/2316	154 (43%)/362	
HR (95% CI)	1.00	2.02 (1.89–2.16)	2.43 (2.25–2.63)	3.20 (2.72–3.77)	<.001
P-value		<.001	<.001	<.001	
HR* (95% CI)	1.00	1.49 (1.39–1.60)	1.68 (1.55–1.82)	2.03 (1.73–2.40)	<.001
P-value*		.001	<.001	<.001	

Hazard ratio (HR) and 95% CI, relative to metabolic score ≤2/5, for each MetRS category.
*HR adjusted for age, sex, and smoking with corresponding *P*-value.
†*P* value for linearity.

(1.07–1.71, $P < .012$) compared to those with the lowest score (GRS ≤1). The multivariable adjusted HR was 14.35 (1.04–1.74, $P = .024$) for those in the highest versus lowest 3-SNP GRS category. The hazard ratio for individuals in the highest MetRS (5/5) category was 3.20 (2.72–3.77, $P < .001$) compared to those in the lowest category (≤2/5). The multivariable adjusted HR was 2.03 (1.73–2.40, $P < .001$) for those in the highest versus lowest category.

The 3-SNP GRS score ≥ 4/6 was associated with an increased risk for CVD independent of the presence of MetRS as the distribution of MetRS amongst each of the GRS categories was similar (Table 8 and Fig. 1). On the other hand, the presence of the MetRS predicts CVD independent of the 3-SNP GRS. Whereas in the participants with the metabolic syndrome (MetRS ≥3/5) an increased CVD risk was observed in all GRS groups that was not the case in participants without the metabolic syndrome (MetRS ≤2/5). CVD HR ranged from 2.15 (1.13–4.09) to 1.70 (1.02–2.83) for participants with a GRS of 0/6 and ≥ 4/6, respectively (Table 9).

Discussion

Evidence on the causal link between elevated plasma TG levels and risk of CVD has recently emerged.[33–35] It is however still under debate what determines the contribution of elevated plasma TG levels to increased CVD risk. Is the presence of the metabolic syndrome leading to elevated plasma TG levels or is genetic predisposition also important for increased TG-mediated CVD risk?

Previous GWAS have discovered multiple genetic loci associated with plasma TG, which have been recently confirmed by a large meta-analysis in >100,000 subjects, detecting 32 loci harboring common variants that contribute to plasma TG concentration.[35] The effect sizes of most of these loci is small but robust as illustrated by Mendelian randomization studies.[36] Here, we demonstrate in the EPIC-Norfolk cohort that both a minimal 3-SNP GRS and MetRS scores were in a stepwise manner associated with plasma TG levels and future CVD risk. To generate a GRS we aimed to find the combination of SNPs, resulting in a 3-SNP GRS by using SNPs *in LPL* and *APOA5*, 2 major genes with a pivotal role in TG metabolism. There was an association between the number of TG-raising alleles and the plasma TG concentration, with a 0.14 mmol/L per allele change. This effect was independent from other known risk factors, which influence plasma TG levels such as age, sex, diabetes, and BMI. No adjustment for HDL-C was performed because of the high degree of correlation with plasma TG. In Mendelian randomization studies focused on TG and remnant cholesterol, a similar observation was found, again suggesting that genetic predisposition results in increased TG levels and CVD risk.[36] Our findings are in line with these observations. Elevated plasma TG, reflected by a 3-SNP GRS ≥ 4/6, is associated with a CVD risk increase, with an HR of 1.35 (95% CI: 1.04–1.74; $P = .024$) compared to those with the lowest 3-SNP GRS.

The MetRS is associated with an increase in plasma TG concentration of 0.72 mmol/L per MetRS point increase, whereas the multivariate adjusted hazard ratio (95% CI) for CVD was 2.03 (1.73–2.40; $P < .001$) for the

Table 8 Overlap between the MetRS and 3-SNP GRS

3-SNP GRS	0/6	1/6	2/6	3/6	≥4/6
MetRS					
≤2/5	119 (75.8%)	2062 (69.4%)	8740 (65.8%)	2050 (60.4%)	142 (53.0%)
3/5	27 (17.2%)	576 (19.4%)	2790 (21.0%)	816 (24.0%)	74 (27.6%)
4/5	10 (6.4%)	287 (9.7%)	1517 (11.4%)	454 (13.4%)	48 (17.9%)
5/5	1 (0.6%)	46 (1.5%)	237 (1.8%)	74 (2.2%)	4 (1.5%)
Total	157 (100%)	2971 (100%)	13,284 (100%)	3394 (100%)	268 (100%)

Data are presented as number (percentage). 3-SNP GRS category = 3-single nucleotide polymorphism gene risk score, MetRS = metabolic risk score.

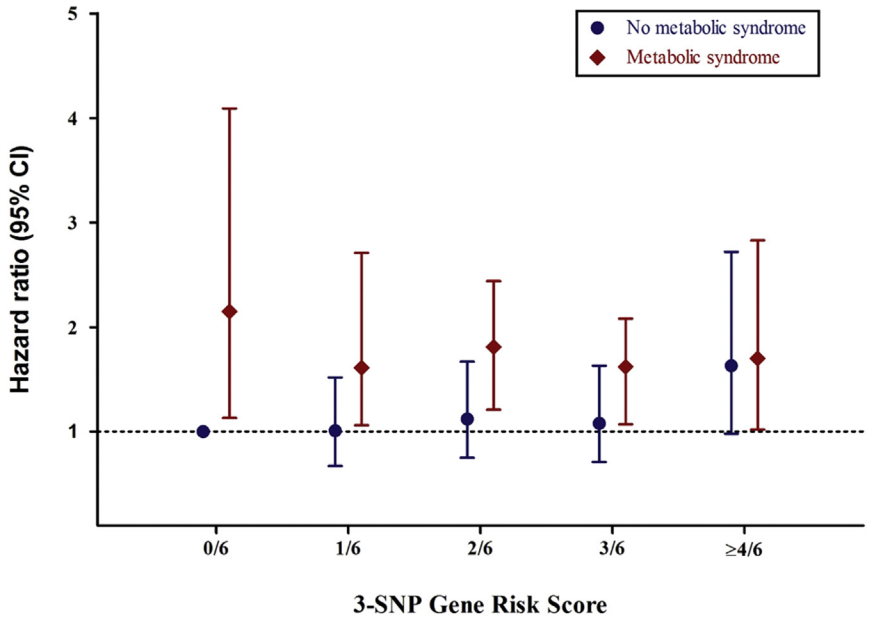

Figure 1 Risk of CVD according to a 3-SNP gene risk score (GRS) stratified for metabolic syndrome risk score (MetRS ≤2 vs ≥ 3).

highest versus the lowest category. An influence of yet undefined genetic (TG related) factors cannot be excluded. Further analysis revealed that the 3-SNP GRS and MetRS both predict TG-mediated CVD risk and are targeting different subgroups with increased CVD risk.

The metabolism of TG-rich lipoproteins, HDL-C, and LDL-C are closely linked. It is therefore interesting to see that together with elevated plasma TG levels, the 3-SNP GRS and MetRS, in the case-control subset study, also coincides with other features of the atherogenic lipid profile such as increased LDL particle number, smaller LDL particle size, increased VLDL particle number reflecting circulating remnant particles, plasma apo A-V levels, and decreased HDL particle size and plasma preheparin LPL mass. It is widely accepted that APO A-V and LPL are pivotal for normal TG homeostasis.[16,17,25] Rare mutations, leading to a loss of function of *LPL*, lead to a severe hypertriglyceridemic phenotype.[48] Of note, a higher 3-SNP GRS and MetRS are associated with lower preheparin LPL plasma levels. Interestingly, this also coincided with elevated plasma apo A-V levels and might explain the observed differences in lipoprotein particle size and increased plasma TG levels due to inhibition of LPL activity by apo A-V. However, it has to be mentioned that the function of apo A-V in lipoprotein metabolism still remains to be established. These changes in lipoprotein composition directly influence its atherogenic properties. Remnant particles may enter the intima of the artery wall and get trapped, which consequently leads to accumulation of remnant cholesterol resulting in foam cell formation.[53]

In conclusion, in the present study, we provide evidence that both the 3-SNP GRS and MetRS scores are significantly associated with increased plasma TG concentrations and an increased risk of CVD.

Table 9 Risk of future coronary artery disease and stroke per 3-SNP GRS and MetRS categories

3-SNP GRS	0/6	1/6	2/6	3/6	≥4/6
MetRS					
≤2/5	1.00 (reference)	1.01 (0.67–1.52)	1.12 (0.75–1.67)	1.08 (0.71–1.63)	1.63 (0.98–2.72)
P-value		.97	.59	.72	.06
≥3/5	2.15 (1.13–4.09)	1.61 (1.06–2.44)	1.81 (1.21–2.71)	1.62 (1.07–2.44)	1.70 (1.02–2.83)
P-value	.020	.025	.004	.022	.041
TG					
<1.7 mmol/L	1.00 (reference)	1.02 (0.67–1.54)	1.12 (0.75–1.67)	1.07 (0.71–1.62)	1.82 (1.06–3.11)
P-value		.37	.60	.75	.03
≥1.7 mmol/L	1.64 (0.86–3.14)	1.34 (0.88–2.03)	1.52 (1.02–2.28)	1.38 (0.92–2.08)	1.43 (0.88–2.34)
P-value	.13	.17	.04	.12	.15

Hazard ratio (95% confidence interval) adjusted for age and sex with corresponding *P*-value. 3-SNP GRS = 3-single nucleotide polymorphism gene risk score, MetRS = metabolic risk score; TG = triglyceride.

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R.V., F.O., R.P.S., S.M.B., E.S.G.S., and G.M.D. performed study design, protocol drafting, statistical analysis, article drafting, and editing. A.H.Z. performed supervision statistical analysis. K.T.K. and N.J.W. performed patient recruitment and data resources.

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S.M.B. has participated in advisory boards for Pfizer and Sanofi-Aventis. E.S.S. reports that his institution has received lecturing fees and advisory boards from Amgen, Regeneron, Sanofi, Ionis, Akcea, Athera.

Supplementary data

Supplementary data related to this article can be found online at https://doi.org/10.1016/j.jacl.2019.02.005.

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